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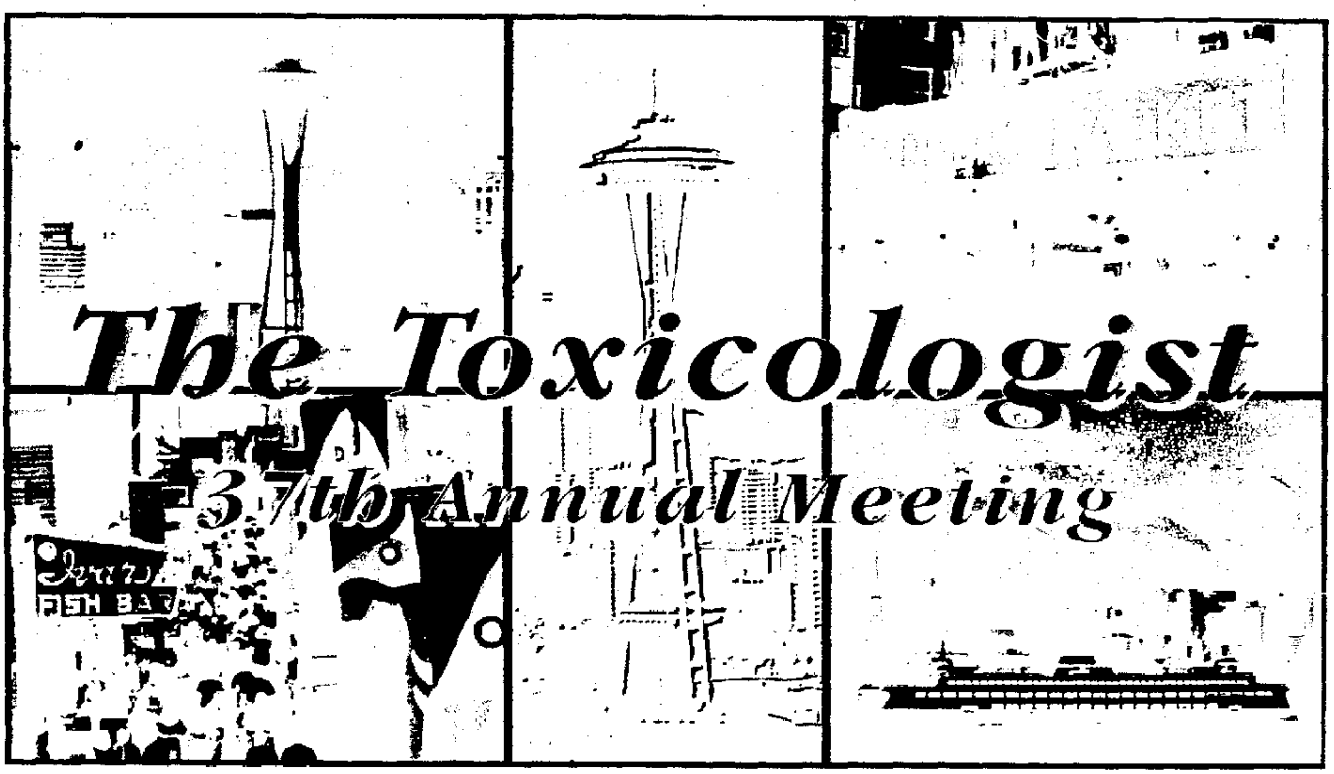

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1449 DISCRIMINATION OF CIGARETTE MAINSTREAM SMOKE CONDENSATES WITH THE SALMONELLA REVERSE MUTATION ASSAY.

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The Salmonella reverse mutation assay has been optimized with regard to reproducibility and discriminative power to investigate the mutagenic activity of the mainstream smoke condensate (MSC) of various research cigarettes. For this purpose, the Kentucky reference cigarette 1R4F along with 57 research cigarette types were smoked according to ISO standards and the MSC was collected by impaction—4 batches for each cigarette type. The condensate was dissolved in dimethyl sulfoxide. The mutagenicity determination was carried out in the plate incorporation version of the Ames assay with *S. typhimurium* strains TA98 and TA100 in the presence of a metabolic promutagen activation system S9. Four equidistant condensate doses ranging from 0.04 to 0.16 mg dry condensate per plate were applied routinely. The specific mutagenicity, i.e. revertants/mg dry condensate, of each cigarette condensate batch was calculated using linear regression analysis with Poisson-weighted data.

Over a period of 4 years, the MSC of 1R4F showed a reproducible specific mutagenicity with a variability coefficient e.g., of 7.4 % in strain TA98. Conventional American blend research cigarettes—differing in cigarette construction, processing aids, and ingredients—varied by less than ± 20 % in their mutagenicity relative to 1R4F. The reconstituted leaf process was found to have no influence on the specific mutagenicity. Non-conventional research cigarette showed a broad range of mutagenicity relative to that of the 1R4F: a pure Burley cigarette showed a relative increase in mutagenicity by 70 %, and an electrically heated cigarette with controlled combustion showed a relative decrease of 90 %.

1450 DISCRIMINATION OF CIGARETTE MAINSTREAM SMOKE WITH THE NEUTRAL RED UPTAKE CYTOTOXICITY ASSAY.

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The neutral red uptake assay has been optimized for the quantitative comparison of the cytotoxicity of the total particulate matter (TPM) and the water-soluble portion of the gas/vapor phase (GVP) of cigarette mainstream smoke. For this purpose, the Kentucky reference cigarette 1R4F along with 23 research cigarettes were smoked according to ISO standards. TPM and GVP were trapped from the same puffs on a glass-fiber filter and in phosphate-buffered saline, respectively, and the TPM was extracted with dimethyl sulfoxide. The cytotoxicity was determined by the uptake of neutral red by BALB/c 3T3 cells which indicates the number of viable cells. The cytotoxicity of each cigarette type was expressed as the reciprocal EC_{50} value calculated using nonlinear regression to the S-shaped logistic function of the concentration response curve.

Over a period of 2 years, TPM fractions of the 1R4F showed a highly reproducible response with a variability coefficient of 8.1 %, whereas GVP fractions varied to a greater extent (21.4 %). To account for this variability, the results of the research cigarettes were normalized to those of the 1R4F. TPM and GVP of conventional American blend research cigarettes—differing in cigarette construction, processing aids, and ingredients—varied by 30 % or less in their relative cytotoxicity. Non-conventional research cigarettes showed a broad range of cytotoxicity relative to the 1R4F: a pure base-web sheet cigarette showed a relative increase in cytotoxicity by 380 %, and an electrically heated cigarette with controlled combustion showed a relative decrease of 80 %.

1451 EQUI-EFFECT DESIGN FOR SUBCHRONIC INHALATION TOXICITY STUDIES COMPARING CONVENTIONAL AND NOVEL CIGARETTE PROTOTYPES.

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Typically, the biological activity of new prototype cigarettes has been estimated by comparing it to that of a reference cigarette at equal total particulate matter (TPM) dose levels.

However, when the TPM and/or gas-vapor phase composition differ significantly and non-proportionally between cigarettes, exposure to equal doses of any one dose parameter (e.g., TPM or puffs) does not result in equal exposure to other dose parameters. This can result in difficulties in data interpretation. To overcome this problem, the concept of exposing animals at equal doses can be replaced by the concept of exposing animals to doses which achieve equal effects. In this concept, the dose ratios for equal effects, i.e., relative biological activities, can be evaluated for each endpoint and dosing parameter of interest, rather than the effect differences at given doses. The two advantages to this concept are: first, the evaluation is independent of the nature of the concentration-response curve (linearity not required), and second, any dose parameter can be used for additional evaluation. An a priori understanding of dose-response characteristics is, however, required. In the present 90-day inhalation study on rats, the biological activity of a novel, electrically heated cigarette prototype with controlled combustion was compared with that of the Kentucky reference cigarette 1R4F. This comparison of such different cigarettes is most logically done on a per cigarette basis. A 3-fold lower relative biological activity was observed for the prototype cigarette compared to the 1R4F. The consistency of the relative biological activities obtained for a large number of endpoints demonstrates the robustness of this study design.

1452 EVALUATION OF AN ELECTRICALLY HEATED CIGARETTE.

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The biological activity of smoke from a novel, electrically heated cigarette prototype is compared to that of conventional cigarettes. This novel cigarette is designed to be used in constant contact with a battery-operated lighter as an external heat source instead of continuously burning tobacco. The lighter consists of a series of eight heater blades which supply computer controlled energy, sufficient to maintain temperature and time limited distillation and pyrolysis without igniting the cigarette.

The cigarette is made of conventional tobacco filler rolled into tobacco mat for optimal heat contact and non-destructive cigarette removal. Due to the enclosed aerosol generation, the new cigarette type generates essentially no sidestream smoke. The mainstream smoke differs substantially in its chemical composition and its biological activity from smoke of the 1R4F Kentucky reference cigarette, which approximates the middle of the US marketplace in tar delivery.

Compared to the yields of the 1R4F, many cigarette smoke constituents are significantly reduced (e.g., total particulate matter by 60%, carbon monoxide by 94%, benzene by 98%, benzo(a)pyrene by more than 98%, tobacco-specific nitrosamines by 83%). Correspondingly, mainstream smoke from the novel cigarette type shows a lower biological potency when compared to the reference cigarette: Bacterial mutagenicity by 97%, in vitro cytotoxicity by 80%, and in vivo irritancy by more than 60%.

1453 ANALYSIS OF ALPHA$_{2\mu}$-GLOBULIN USING LIQUID CHROMATOGRAPHY/ ELECTROSPRAY IONIZATION-MASS SPECTROMETRY (LC/ESI-MS).

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Excessive accumulation of alpha$_{2\mu}$-globulin in hyaline droplets in kidneys of male rats as a result of the formation of a protein-ligand complex with certain types of toxicants is an important biomarker for nephrotoxicity. As a result of the gentle nature of ESI-MS, proteins and protein-ligand complexes have been successfully promoted into the gas phase for subsequent mass spectrometric analysis. We have explored the feasibility of LC/ESI-MS for alpha$_{2\mu}$-globulin analysis using a Finnigan MAT TSQ 7000 triple quadrupole mass spectrometer. The mass spectrometer was initially tuned and calibrated using a solution of apomyoglobin. HPLC-purified alpha$_{2\mu}$-globulin was then infused into the ESI source and the charged-state distribution envelope was obtained. The charged-state distribution envelop revealed a series of ions that could be used for selected reaction monitoring and could be deconvoluted to confirm the molecular mass of alpha$_{2\mu}$-globulin. Urine samples (16-hr) were collected from rats and low molecular weight material was removed using Centricon-10 molecular weight cut-off filters prior to injection of the remaining proteins onto a reversed-phase analytical HPLC column. Proteins were eluted from the column into the ESI source and alpha$_{2\mu}$-globulin was

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